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The Rhesus Monkey: a Primate Model for Hemopoietic Stem Cell Studies

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Abstract. Two heterogeneous cell populations (CP 1–7 and CP 8–10) were separated from rhesus monkey bone marrow using counterflow centrifugation–elutriation (CCE). These two cell populations were distinct with respect to morphological composition, expression of cell surface antigens, hemopoietic progenitor cell activity, and concentration of hemopoietic stem cells (HSC). The hemopoietic progenitor cell activity and HSC were concentrated in CP 8–10. In autologous transplantation studies, CP 8–10 reconstituted the lymphohemopoietic system of lethally irradiated monkeys in a manner similar to that of monkeys transplanted with unfractionated bone marrow cells. CP 1–7 was lymphocyte rich and depleted of progenitor cell activity. Transplantation of CP 1–7 led to eventual lymphohemopoietic reconstitution of irradiated monkeys; however, complete engraftment was delayed by as much as 14 days compared to either the transplantation of CP 8–10 or to unfractionated bone marrow. Thus, a presence of the HSC in the lymphocyte-rich progenitor-cell-depleted population can be detected in the rhesus model. (Reprints)

Key words: Counterflow centrifugation–elutriation (CCE) — Hemopoietic stem cell (HSC) — Rhesus monkey — Hemopoietic progenitor cells

A variety of hemopoietic studies have been pursued in nonhuman primates in an attempt to find a valuable nonhuman model. Several hemopoietic cell-separation techniques have been applied to the bone marrow of the rhesus monkey (*Macaca mulatta*), and results have been compared to those obtained using human bone marrow. Moore et al. [1] used two albumin gradient-density centrifugation steps to obtain a cell population enriched in progenitor cells for granulocyte–macrophage (CFU-c). Rhesus and human bone marrow CFU-c were isolated and enriched in the same light-density region of the gradient. In a further study using albumin gradient centrifugation, the enriched CFU-c fraction from rhesus monkey was evaluated in allogeneic bone marrow transplantations in order to prevent graft-versus-host disease [2]. The transplantation of this bone marrow fraction, however, resulted in a low proportion of bone marrow engraftment.

Another method of bone marrow separation in both nonhuman primates and humans used the lectin, soybean agglutinin, in which the nonagglutinated (or negative) fraction in both species was shown to be enriched in CFU-c activity [3]. The nonagglutinated human marrow fraction was evaluated successfully in clinical allogeneic bone marrow transplantations [4]. A correlation suggests that the hemopoietic stem cell (HSC) would also be found in the nonagglutinated marrow fraction of primates; in fact, a preliminary report supports these findings [5].

Jemionek et al. [6] separated human and rhesus monkey bone marrow using counterflow centrifugation–elutriation (CCE) equipped with a continuous albumin gradient. The elutriation activity profiles for the granulocyte–macrophage colony-forming cell (GM-CFC) was shown to be similar for both rhesus monkey and human bone marrows. Additional studies using CCE separated human bone marrow cells into a population enriched in myeloid progenitor cell activity [7, 8]. This enriched myeloid progenitor cell population showed a similarity to the enriched GM-CFC marrow cells separated from rhesus monkey and human marrow by Jemionek et al. [6]. Based on these reports, there appears to be a relationship between the elutriation position of both rhesus monkey and human marrow GM-CFC activity.

Although bone marrow cell separation techniques and isolated progenitor cell activities in nonhuman primates and humans have been compared, an extension of this comparison to include func-

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tional hemopoeitic reconstitution in vivo has not been reported. The studies presented in this paper use the rhesus monkey to study HSC physiology in the hope that information obtained from this model might be extrapolated to the human. The objective was to use CCE to rapidly separate various bone marrow cell populations. The separated cell populations were identified morphologically, evaluated against antihuman monoclonal antibodies, and cultured in vitro for progenitor cell activity. In addition, the pluripotent HSC content of these cell populations was assayed by testing for their ability to reconstitute the lymphohemopoeitic systems in an autologous bone marrow transplantation model.

Materials and methods

Monkeys

Normal male rhesus monkeys, *Macaca mulatta*, weighing 6–9 kg and born and raised in the USA, were used. All technical manipulations of the animals were performed with the monkeys under anesthesia (ketamine, 10 mg/kg, i.m.). Monkeys undergoing bone marrow aspirations or used as plateletpheresis donors were further anesthetized with Biotal (4% sodium thiamylal, 10 mg/kg; Bioceutic Labs, St. Joseph, Missouri). All monkeys were under the care and supervision of the veterinary staff, and research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

Bone marrow aspirations

Both iliac crest areas were shaved and prepared aseptically, and marrow was collected from the posterior horn of the iliac crest with multiple aspirations, using an 18-gauge Rosenthal needle (Becton-Dickinson, Mountain View, California) and a 10-cm³ syringe with preservative-free heparin (500 U). Enough bone marrow was aspirated to yield between 300 and 600 × 10^6 cells. Aspirated bone marrow was washed twice with Hanks' balanced salt solution minus calcium and magnesium (HBSS−), resuspended to twice the original volume with HBSS−, and then centrifuged (400 *g* for 30 min) over a layer of lymphocyte separation medium (Litton Bionetics, Rockville, Maryland). All cells above the red blood cell pellet were recovered, washed twice with HBSS−, and resuspended in 40 ml of elutriation medium. This sample was identified as the *pre* sample. Bone marrow cells used in in vitro studies were also obtained by flushing cells from rib sections obtained from healthy donors killed for organ procurement (Flow Labs, McLean, Virginia).

Counterflow centrifugation-elutriation (CCE)

The study was divided into two phases. The first involved determining the elutriation position of specific cell populations and in vitro culture activities of the hemopoietic progenitor cells. The second phase used an abbreviated elutriation procedure to separate the marrow into two cell populations.

CCE was performed as described by Jemmack et al. [17] with the following modifications: The initial parameters for the run were a centrifuge speed of 2000 rpm, a temperature of 18°C, and a flow rate of 7.5 ml/min. Marrow *pre* sample cells were first allowed to enter the chamber, and then were separated by increasing the flow rate in a sequence defined by the curve in Figure 1. A total of 16 40-ml fractions were collected. After the 14th fraction, the centrifuge was turned off while the flow rate was held constant. The cells from each fraction were collected by centrifugation. Fractions 15 and 16 were pooled as the rotor-off fraction.

In the second phase of the CCE studies, the elutriation run was abbreviated. The flow rates for the first eight fractions were the same as in phase 1; then the centrifuge was turned off at fraction 9, collecting through fraction 10. Fractions were centrifuged, cells resuspended, and fractions 1–7 and 8–10 were pooled, respectively.

Cellular analysis

Cell counts were determined using Coulter Counter model ZBI. Cytospin cell preparations were made for each elutriation bone marrow fraction and stained with Wright–Giemsa solution; 500 cell-count bone marrow differentials were made on each fraction. Hematology reports on peripheral blood samples were accomplished using Coulter Counter model S Plus II. Differentials were made on the peripheral blood smears.

Antigenic determinations

Monoclonal antibody analysis. Fluorescein conjugates of Leu 2a (suppressor/cytotoxic), Leu 3a/b (helper/inducer), and HLA-DR (Becton-Dickinson) were used for analyses of peripheral blood and bone marrow cells. Procedures used for cellular preparations and monoclonal labeling have been described by Hale and McCarthy [9]. Analyses of the samples were performed on either the FACS analyzer or on the FACS II system.

Sheep red blood cell E-rosette analysis. The preparation of neuraminidase-treated sheep red blood cells (S-RBC) and the procedure for E-rosette analysis were as described by Weiner et al. [10]. After the cold incubation, the cell pellet was gently disrupted, and positive cell counts were made using a hemocytometer. The criterion for an E-rosette-positive cell was that it have four or more S-RBC attached to it.

In vitro culture progenitor cell analysis

GM-CFC and CFU-MK culture techniques. The double-layer, soft agar culture technique was used to assay for both GM-CFC and CFU-MK (megakaryocyte progenitors) [11]. Colony-stimulating activity (CSA) was added to the lower agar layer. The source of CSA for GM-CFC was giant-cell-tumor-conditioned medium (GCTCM) (Gibco, Grand Island, New York) used at 10% (vol/vol). CFU-MK expression required GCTCM (7% vol/vol) plus human spleen-conditioned medium (HUSCM) (7% vol/vol) and normal human plasma (5% vol/vol). HUSCM was prepared using the technique described in Ref. [12]. Cultures were incubated at 37°C in an atmosphere of humidified air containing 5% CO_2. GM-CFC-derived colonies (> 50 cells) were counted on day 10 of culture. CFU-MK cultures were incubated for 10–12 days. After this, the upper agar layer was fixed and dried. CFU-MK colonies (> 50 cells) were enumerated after staining with Wright-Giemsa solution.

CFU-e, BFU-e, and CFU-mix culture techniques. Erythroid burst-forming units (BFU-e), erythroid colony-forming units (CFU-e), and mixed cell colony-forming units (CFU-mix) were determined using a plasma clot culture system described in detail by Weinberg et al. [13] Bone marrow cells (5×10^5 cells/ml) were plated as 0.1-ml clots for CFU-e and BFU-e. Cells ($2.5–5.0 \times 10^5$ cells/ml) were plated as 0.4-ml clots for CFU-mix. Anemic sheep plasma, step III erythropoietin (Connaught Labs), was added to culture media for CFU-e (0.5 U/ml), BFU-e (2.0 U/ml) and CFU-mix (1.0 U/ml). Lymphocyte-conditioned medium, 5% (vol/vol), was added to the culture media for CFU-mix. Lymphocyte-conditioned medium was prepared as described by Ash et al. [14], except that a pool of three rhesus monkey peripheral blood mononuclear cells was used as the lymphocyte source. Plasma-clot cultures were harvested on day 4 for CFU-e, on days 8–9 for BFU-e, and on days 11–14 for CFU-mix. Clots were fixed, stained, and evaluated as described previously [14–16].

Transplantation

Monkeys, recipients of autologous bone marrow transplantation, had their bone marrow removed and recovered from anesthesia before being irradiated. They were placed in a Plexiglas chair for irradiation, and exposed bilaterally, using two equivalent cobalt-60 sources. A midline tissue dose of 9.0 Gy was delivered at a dose rate of 0.1 Gy/min. Bone marrow cells were transfused intravenously through the saphenous vein within 1–2 h after irradiation exposure.

Preliminary peripheral blood samples used to establish a hematological baseline were drawn from the monkeys over a two-week period prior to irradiation. Mean irradiation control values were determined and represented 100% levels. The mean of the preirradiation peripheral blood granulocyte levels for all experimental animals was 4.12×10^9/ml ± 0.43. After irradiation and transplantation, the animals were clinically evaluated; hematological parameters were monitored, fluids administered as clinically warranted, and antibiotic treatment (gentamycin sulfate [3 mg/kg/day, i.m.]) was administered prophylactically (beginning on day 5 and continuing until the granulocyte count was maintained above 1000/mm³). Platelet concentrates irradiated with 50.0 Gy cobalt-60 were administered on days 8, 11, 14, and 17. Recovery and a sustained level of the peripheral blood granulocytes and platelets were used to indicate engraftment of the transplanted bone marrow cells.

Results

Recovery of nucleated bone marrow cells

The initial objective of the phase 1 studies was to establish a rapid and reproducible physical separation technique for separating cell populations containing hemopoietic progenitor cells (GM-CFC, CFU-e, CFU-MK). Nucleated bone marrow cells, $300–600 \times 10^6$, were separated over 16 fractions, using CCE, in less than 60 min, with a cell recovery of 86.3% and a viability greater than 98%. The nucleated cell recovery profile (Fig. 1) obtained by eluting the cells from the chamber with increasing flow rates was shown to be reproducible, with only 5%–10% of the nucleated cells being recovered in any

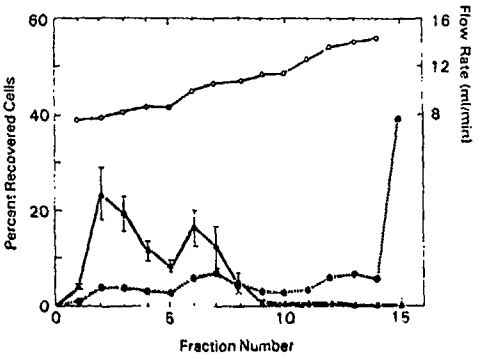

Fig. 1. Cellular elutriation characteristics of aspirated rhesus monkey bone marrow. A graphic presentation of flow rate (O) schedule used per fraction. Recovery of nucleated cells (●) per fraction expressed as percentage of total number of nucleated cells recovered. Recovery of lymphocytes (▲) per fraction expressed as percentage of total number of lymphocytes recovered.

of the fractions until fraction 15. Fraction 15 had the highest concentration of cells, and it represented the chamber contents when the rotor was turned off. In several experiments (data not shown), elutriation was continued through 20 fractions and a final flow rate of 20 ml/min, but the nucleated-cell profile was still relatively unremarkable. Thus, the existing 16-fraction procedure was followed. Morphological identification of cells in each fraction resolved the characteristic elutriation position of specific bone marrow cells. For example, lymphocytes were separated into a biphasic elutriation profile with peak cell concentrations at fractions 2 and 6 (Fig. 1). Other morphologically identifiable bone marrow cells (e.g., normoblasts, myelocytes, monocytes) also had characteristic and reproducible elutriation positions.

Recovery profiles of hemopoietic progenitor cells

Analysis of each fraction for hemopoietic progenitor cell activities (GM-CFC, CFU-e, and CFU-MK) resulted in the total-activity-recovered profiles depicted in Figure 2. The first elutriation fraction that contained any progenitor cell activity (GM-CFC and CFU-MK) was fraction 5. GM-CFC activity was recovered in every fraction after fraction 5, but in any one fraction, with the exception of fraction 15, only 1%–8% of the total activity was contributed. Fraction 15 contained 43.4% of the recovered GM-CFC activity. The CFU-MK elutriation profile was similar to that of GM-CFC, except that more of the activity (58%) was found in fraction 15. Almost all of the CFU-e activity was confined to fraction 15.

The results of the phase 1 studies led to the fol-

Table 1. Expression of cell surface antigens on rhesus monkey peripheral blood mononuclear cells and bone marrow cells

Sample	(*n*)	Leu 2A[a]	Leu 3 A/B	HLA-DR	S-RBC[b]
PB[c]	(11)	34.6 ± 2.6	33.5 ± 1.7	8.7 ± 0.9	74.6 ± 9.0
Pre	(7)	11.1 ± 3.0	11.7 ± 3.7	7.9 ± 1.3	11.7 ± 1.7
CP 1–7	(7)	18.7 ± 3.6	19.5 ± 4.5	7.8 ± 2.4	51.8 ± 4.7
CP 8–10	(7)	1.7 ± 0.5	1.9 ± 0.7	7.0 ± 2.4	2.9 ± 0.8

[a] The mean ± SEM are listed, expressed as the percentage of total population evaluated. All marrow cell data are based on aspirated marrow.

[b] E-rosette receptor with neuraminidase-treated sheep red blood cells.

[c] Peripheral blood mononuclear cells.

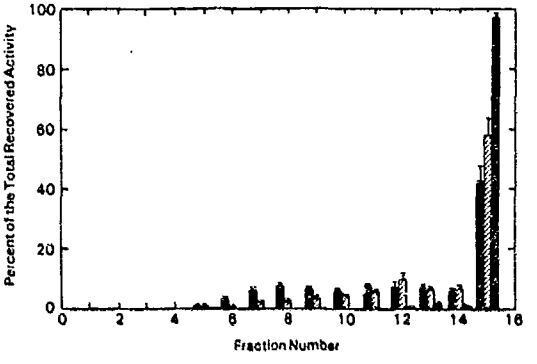

Fig. 2. Elutriation characteristics of progenitor cell activities. Recovery of progenitor cell activities (GM-CFC, ■; CFU-MK, ▨; and CFU-E, ▤) per fraction expressed as percentage of total progenitor cell activity recovered.

lowing observations: (a) more than 99% of the lymphocytes were elutriated by fraction 10, (b) the progenitor cell activity was not observed until fraction 5, and (c) specific, morphologically identifiable cell types were elutriated in a characteristic and reproducible manner. At this point, a decision was made (based on the above observations) to separate the fractioned bone marrow into two cell populations. The first cell population was enriched by lymphocytes and contained a small percentage of the progenitor cell activity. This cell population was composed of fractions 1–7 (CP 1–7). It contained 94.4% of the recovered lymphocytes, 32.9% of the recovered bone marrow cells, and only 9.9% of the recovered GM-CFC activity. The second population was composed of fractions 8–15 (CP 8–10). It contained 67.9% of the recovered bone marrow cells, 90.1% of the recovered GM-CFC activity, 96% of the recovered CFU-MK activity, all of the CFU-e activity, and only 5.6% of the recovered lymphocytes.

Phase 2: Characterization of CP 1–7 and CP 8–10

Morphology. Phase 2 studies focused on the characterization of the two populations identified in phase 1. The cellular composition of CP 1–7 contained predominantly lymphocytes (75.2%), with a smaller number of normoblasts (14.8%), monocytes (5.0%), and only a few myeloid cells (band and segmented forms). CP 1–7 did not contain blast forms of any cell lineage and no mitotic cells were observed. In contrast, CP 8–10 was predominantly composed of mature myeloid cells (69.5% band and segmented forms). The other major cell types in this cell population were normoblasts (10.6%), lymphocytes (7.8%), and monocytes (4.8%). In addition, CP 8–10 contained blast forms of all cell lineages, as well as mitotic cells.

Cell surface antigens. Cell surface antigens of cells from the peripheral blood, *pre* bone marrow, CP 1–7, and CP 8–10 bone marrow fractions are compared in Table 1. Comparisons of the cells of the *pre* marrow with those of the peripheral blood showed that approximately one-third fewer E-rosette-, Leu 2a-, and Leu 3a/b-positive cells existed in the bone marrow. By contrast, the levels of HLA-DR-positive cells in the bone marrow and in the peripheral blood were approximately equal. Comparison of the separated bone marrow cell populations, CP 1–7 and CP 8–10, showed that the cell surface antigens characteristic of lymphocytes (S-RBC E-rosette, helper-inducer, and suppressor-cytotoxic) were concentrated in CP 1–7. This was consistent with the morphological composition of CP 1–7. The HLA-positive cells were distributed equally in both populations.

Hemopoietic progenitor cell activities. Hemopoietic progenitor cell activities of CP 1–7 and CP 8–10 are presented in Table 2. The two populations exhibit quite different activities, with almost all of the hemopoietic progenitor cell activity recovered in CP 8–10. BFU-e, CFU-e, and CFU-mix activities were enriched in CP 8–10, recovering 207.5%, 128.2%, and 125.9% of the *pre* activities, respectively. The

Table 2. Progenitor cell activity recovered in isolated bone marrow populations[a]

Sample	GM-CFC (n = 8)	CFU-e (n = 9)	BFU-e (n = 6)	CFU-mix (n = 4)
Pre	100.0	100.0	100.0	100.0
CP 1-7	5.6 ± 2.5	1.7 ± 1.2	0.2 ± 0.2	3.6 ± 3.1
CP 8–10	90.3 ± 15.8	128.2 ± 56.0	207.5 ± 49.9	125.9 ± 45.2

[a] Mean ± SEM are expressed as the percentage of total preactivity recovered.

GM-CFC activity, although not enriched in CP 8–10, was also concentrated in CP 8–10.

Bone marrow transplantation. Autologous bone marrow transplantation was used as an assay for the presence of the pluripotent HSC. Two control monkeys that had been irradiated with 9.0 Gy and given therapeutic support, but not transplanted with autologous bone marrow, survived only 11 and 13 days. These monkeys did not show signs of hemopoietic recovery and had aplastic bone marrow upon necropsy. Monkeys that had been transplanted with autologous unfractioned (*pre*) bone marrow cells (cell doses of 26.1, 29.9, and 80.2 × 10^6 cells/kg) all survived. The peripheral blood granulocyte recoveries that were used to monitor bone marrow engraftment in these animals increased to at least 20% of control levels by days 16–22 after transplantation (Fig. 3). Granulocyte levels increased continuously through 100% control values before reaching a plateau and gradually returning to normal values by day 55. The remaining peripheral blood cells (platelets, lymphocytes, and monocytes) recovered to normal values by approximately day 55 after transplantation.

Two out of three monkeys survived in experiments in which monkeys were transplanted with CP 1–7. The monkey that did not survive died on day 14, did not exhibit a recovery in its peripheral blood granulocytes, and had aplastic marrow. The two surviving monkeys were transplanted with 31.4 and 37.8 × 10^6 nucleated cells/kg, whereas the monkey that died was transplanted with only 21.1 × 10^6 nucleated cells/kg. Granulocyte recoveries of the two surviving monkeys were biphasic and less rapid than those of monkeys transplanted with unfractionated marrow (Fig. 4). In fact, monkeys that had been transplanted with CP 1–7 reached the 20% control level at days 22 and 24, but then maintained a plateau level between 11% and 27% of control for the next 11 days. After days 33–35, the peripheral blood granulocytes began to recover to normal values at a rate similar to that of monkeys transplanted with unfractionated bone marrow. The other peripheral blood cells recovered to normal levels after the granulocytes had recovered.

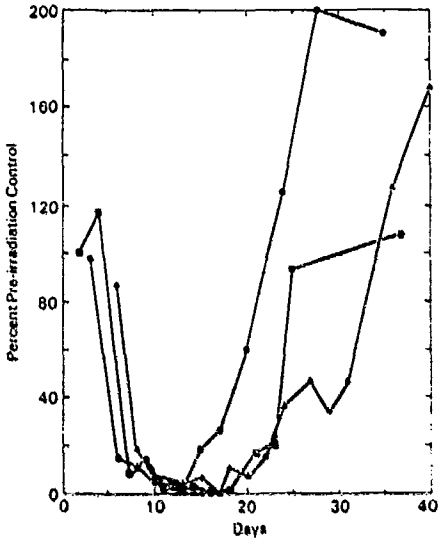

Fig. 3. Rhesus monkey peripheral blood granulocyte recovery curves after a 9.0-Gy dose of irradiation and autologous transplantation of unfractionated bone marrow. Monkeys were transplanted with 80.2 (●), 39.9 (■), and 26.6 (▲) × 10^6 nucleated cells/kg. Granulocytes are expressed as percent of preirradiation control level found in peripheral blood.

All monkeys that had been transplanted with CP 8–10 survived. The monkey that had been transplanted with the lowest number of nucleated cells (23.3 × 10^6 nucleated cells/kg) showed the slowest rate of granulocyte recovery, not reaching the 20% control level until day 27 (Fig. 5). The other two monkeys that had been transplanted with 35.5 and 25.5 × 10^6 nucleated cells/kg showed granulocyte recoveries to the 20% control point on days 20 and 18, respectively. The granulocyte recoveries of all monkeys transplanted with CP 8–10 continued to increase steadily through the 100% level. As was observed with the other transplanted monkeys, the remaining peripheral blood cells recovered to normal values after the granulocytes did so.

Discussion

Two cell populations were separated from rhesus monkey bone marrow with a rapid, gentle, and re-

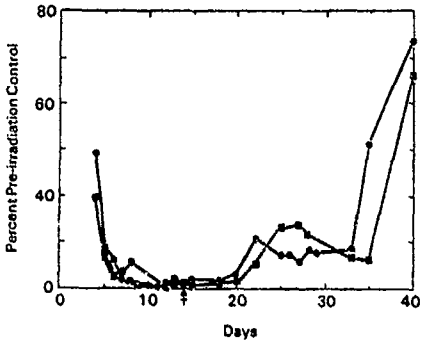

Fig. 4. Rhesus monkey peripheral blood granulocyte recovery curves after a 9.0-Gy dose of irradiation and autologous transplantation of CP 1–7. Monkeys were transplanted with 37.8 (●), 31.4 (■), and 21.1 (▲) × 10^6 nucleated cells/kg. Granulocytes are expressed as percent of preirradiation control level found in peripheral blood.

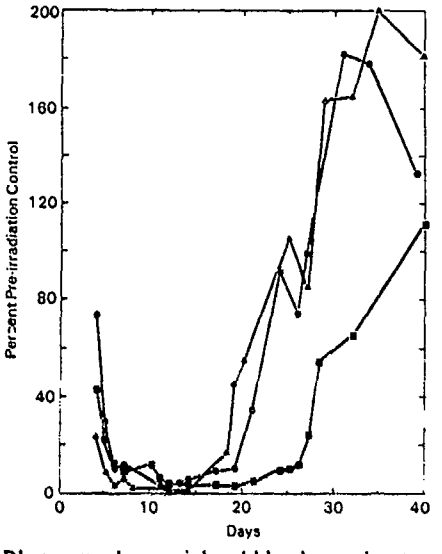

Fig. 5. Rhesus monkey peripheral blood granulocyte recovery curves after 9.0 Gy and autologous transplantation of CP 8–10. Monkeys were transplanted with 35.5 (●), 23.3 (■), and 25.0 (▲) × 10^6 nucleated cells/kg. Granulocytes are expressed as percent of preirradiation control level found in peripheral blood.

producible procedure using the technique of counterflow centrifugation–elutriation (CCE). These two cell populations, designated CP 1–7 and CP 8–10, differed in their proportions of specific cell types and of in vitro colony-forming hemopoietic progenitor cells (GM-CFC, CFU-e, BFU-e, and CFU-mix). CP 1–7 was composed primarily of lymphocytes, and contained less than 6% of the recovered hemopoietic progenitor cell activity. In contrast, CP 8–10 contained almost all the recovered hemopoietic progenitor cell activity, and was composed predominantly of myeloid cells, with only a minor lymphocyte contribution.

The potential of these two cell populations to reconstitute the hemopoeitic system of lethally irradiated autologous recipients served as an assay for the pluripotent hemopoietic stem cell (HSC). After transplantation of either CP 1–7 or CP 8–10, there was an initial increase in the recovery of peripheral-blood granulocytes in monkeys on days 20–25. However, the granulocyte levels of monkeys transplanted with CP 8–10 reached above 100% control levels before achieving a plateau. The granulocyte recovery profiles of these monkeys were similar to those obtained from monkeys transplanted with unfractionated bone marrow. In contrast, there was a biphasic recovery of granulocytes in monkeys transplanted with CP 1–7. A plateau level of 11%–27% of control values was reached and maintained for 11 days (from day 22 to day 33). On days 33–35, there was an increase in granulocytes to control levels. Thus, the transplantation of CP 1–7 led to a slower rate of complete engraftment. The eventual recovery of monkeys transplanted with CP 1–7 can be attributed to the possibility that CP 1–7 contained a small proportion of HSC. In two of the cases, there were enough stem cells to enable engraftment, although delayed. In the third monkey, there were possibly not enough HSC in the cells transplanted to enable engraftment, and death resulted. From the data, we conclude that CP 8–10 demonstrated a greater potential than CP 1–7 in restoring hemopoiesis, and therefore contained a higher concentration of the HSC. In addition, we have demonstrated, using the rhesus monkey model, that there was a good correlation between the in vitro hemopoietic progenitor cell activity and the presence of the HSC.

There appear to be differences in the elutriation position of the HSC in the various large-animal models used in hemopoietic studies. Jemionek et al. [17] isolated three fractions (I, II, and III) in the elutriation of dog bone marrow. In those studies, fraction I was the only fraction shown to have the capability to reconstitute the hemopoietic system of a lethally irradiated dog. The elutriated cell populations, CP 1–7 and CP 8–10, of rhesus marrow were similar both in morphology and progenitor cell activities to dog marrow fractions I and III, respectively. However, unlike the dog model, CP 8–10 (dog fraction III) had the greatest capability to reconstitute the hemopoietic system of a lethally irradiated monkey, while CP 1–7 (dog fraction I) had a lesser capability.

The results presented here for CP 8–10 further expand on the elutriation survey of mammalian bone marrow by Jemionek et al. [6]. In their studies, the GM-CFC activities of both monkey and human bone marrow were elutriated in the larger cell population

of predominantly myeloid cells. De Witte et al. [7], using a combination of Percoll density-gradient centrifugation and CCE, separated cells from human bone marrow that had characteristics similar to those of CP 8–10, as described in the present study. These similarities included enrichment of myeloid progenitor cell activities, composition primarily of immature myeloid cells, and only minor lymphocyte contamination. The isolated human bone marrow cells were evaluated clinically and transplanted into allogeneic bone marrow recipients (HLA-B, C, and D identical siblings). These transplanted donor cells engrafted, and there was a recovery of donor peripheral blood cells in the recipients [18]. Therefore, this separated human cell population contained the necessary HSC to lead to engraftment even in an allogeneic donor–recipient combination. CP 8–10 of the rhesus monkey bone marrow has been shown in this paper to contain a higher proportion of the recovered hemopoietic progenitor cell activity and a concentration of HSC sufficient for hemopoietic reconstitution of a lethally irradiated recipient. Thus, CP 8–10 appears to be analogous to the cell populations separated and transplanted in human transplant studies described by De Witte et al. [7, 18]. A recent report on human bone marrow by Noga et al. [8] further demonstrates the separation of lymphocytes from in vitro hemopoietic progenitor cell activity and immature myeloid cells using CCE.

These comparative reports demonstrate that the progenitor cells and the HSC of the rhesus bone marrow have physical separation characteristics similar to those of cell populations found in human marrow. In addition, the rhesus HSC was concentrated in cell populations that were similar to that of the human HSC, based on morphological and antigenic comparison. In contrast, it was also possible to demonstrate the presence of HSC in the rhesus lymphocyte-rich, progenitor-cell-depleted cell population (CP 1–7); whereas in the human situation, this type of analysis has been impossible to accomplish.

The high degree of cross-reactivity to antihuman monoclonal antibodies to the cell surface antigens on rhesus lymphoid [19–21] and hemopoietic [22, 23] cell types allows this animal to be a useful nonhuman model for the study of hemopoiesis. In the rhesus model, cellular and humoral regulatory requirements of the HSC can be evaluated in vitro concurrently with studies of humans, using available human reagents. A further advantage of the rhesus bone marrow transplantation model is that these requirements can also be evaluated in vivo. Evaluations of this type can address questions on the optimal number of particular cell populations necessary for successful engraftment in the autologous transplantation model. This, in turn, can be extended to the more complex questions associated with the allogeneic situation.

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